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MRSA screening: can one swab be used for both culture and rapid testing? An evaluation of chromogenic culture and subsequent Hain GenoQuick PCR amplification/detection

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Abstract

The use of a single swab for both MRSA culture and for rapid testing by PCR was evaluated, using the Hain GenoQuick (GQM) methicillin resistant *Staphylococcus aureus* (MRSA) assay for the rapid detection of MRSA, as a single swab would be the preferred option for routine diagnostic testing. GQM detected current prevalent Irish MRSA strains incorporating all known *SSCmec* types, including Panton–Valentine leukocidin-positive strains. Using the GQM method, all methicillin-resistant coagulase-negative staphylococci tested were confirmed to be negative, although three of seven gentamicin-resistant methicillin-sensitive *Staphylococcus aureus* strains tested were identified as MRSA. The theoretical *ex-vivo* limit of detection of the assay was 704 CFU per GQM assay reaction (1.7×10^4 CFU/mL) when MRSA suspensions were used for DNA extraction, or 1.4×10^3 CFU/swab (1.4×10^4 CFU/mL) using MRSA absorbed onto Copan screening swabs. Swab processing on chromogenic agar prior to PCR resulted in some inhibition of the PCR reaction, increasing the limit of detection of the assay by a factor of four. Based on 540 single swab screening specimens (nasal and groin) processed first for culture assay, then by GQM, the specificity and positive predictive value were both 100%, the negative predictive value was 92%, and the sensitivity was 57%. Culture followed by PCR from a single specimen is not optimal for the rapid detection of MRSA. Further laboratory validation of the GQM assay is required to determine the true diagnostic sensitivity and value of this kit in routine microbiology laboratories, modifying the protocol for single specimens, or using two specimens.

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Introduction

A major component in the control of methicillin-resistant *Staphylococcus aureus* (MRSA) transmission is the early detection of patients either colonized or infected with MRSA, followed by isolation to prevent cross-infection [1]. Several studies evaluating the accuracy of various MRSA detection kits (IDI MRSA: Cephid, Maurens-Scopont, France; GeneXpert MRSA: Cephid, Sunnyvale, CA, USA; GenoType MRSA: Main Life Science, Nehren, Germany; and the MRSA EVIGENETM: Statens Serum Institut, Copenhagen, Denmark) and PCR in-house assays [2–8] have been published. The Hain GenoQuick (GQM) MRSA assay (Hain Lifescience, Nehren, Germany) is a new molecular assay. Its predecessor, GenoType MRSA, is a PCR assay using DNA Strip technology for amplicon detection, and permits the identification of *S. aureus*, *S. epidermidis*, and the *mecA* gene, with a specimen turn-around time of *c.* 7 h [7,9]. GQM is based on the same immuno-chromatographic detection technology, but detects MRSA with a reported turn-around time of only *c.* 2.5 h. MRSA-specific chromosomal sequences are targeted. The assay consists of three steps: direct DNA extraction from a patient swab using a lysis buffer provided, followed by

multiplex PCR (primer/probe/nucleotide mix provided) with single-stranded amplicon hybridization using a specific probe, and finally, the detection of amplicon/probe complex—the complex selectively binds to the test band on the dipstick and is visualized by gold labelling. Each dipstick includes two control zones: a conjugate control zone to check the binding of the conjugate on the dipstick and an amplification control zone to check for a successful amplification reaction.

Here we determined the accuracy and the limit of detection (LoD) of the GQM assay and established the potential to use one MRSA screening swab for two methods of MRSA detection. This specimen process flow is preferred where diagnostic laboratories choose to continue culture with PCR for confirmation, and to use the isolate for epidemiological purposes. Additionally, this flow enables adherence to accredited laboratory standard operating procedures, minimizing the additional nursing, laboratory and administrative workloads, and the financial burden associated with taking and processing two specimens.

Methods

Bacterial isolates

The capacity of the GQM assay to detect Irish MRSA strains (n = 32) was assessed using a collection provided by the National MRSA Reference Laboratory, Dublin and Dublin Dental School and Hospital, Trinity College Dublin [10,11]. All isolates were tested, according to manufacturer's instructions, for pure culture analysis. This isolate group comprised 28 isolates representative of common epidemiological types recovered in Ireland and four control strains, including the following SCC*mec* types (I, IA, II, IIA–IIE, III, IIIv, IV and IVa–IVh, V, VI).

Two reference strains, MRSA-ATCC 43300 and methicillin-sensitive *Staphylococcus aureus* (MSSA)-ATCC 29213, were included. A further seven gentamicin-resistant methicillin-susceptible *Staphylococcus aureus* (GrMSSA) strains [12] and eight various methicillin-resistant coagulase-negative staphylococci (MRCNS) were also tested.

Conventional culture and MRSA identification

MRSA screening specimens were collected using single Copan 151C cotton albumin coated swabs (Medical Supply Company, Dublin, Ireland) as per laboratory standard operating procedures. The GQM assay does not specify what type of swab is optimal for use. Swabs were assessed for the presence of MRSA by plating on MRSA Select chromogenic media (CA) for the isolation and identification of MRSA (Bio-Rad Life Science Group, Marnes-la-Coquette, France). Colonies deemed positive on this media were subcultured on Columbia Blood Agar (CBA) (Cruinn, Ireland) and confirmed by slide coagulase (Staphaurex Plus, Remel; Oxoid Ltd., Basingstoke, UK) and by automated antibiotic susceptibility testing (Phoenix, BD Biosciences, Pharmingen, CA, USA).

Limit of detection assays

LoD assays (n = 3) of the GQM assay were performed as follows: A 0.5 M^{c}Farland of strain MRSA ATCC 43300 was prepared in saline and a ten-fold dilution series was made. CFU per millilitres was determined by spread plating each dilution on CBA and incubating at 37°C for 24 h.

To calculate the LoD of the assay when MRSA suspensions were used directly for DNA extraction protocols, 40 μL of each dilution above was added to 260 μL of buffer Q-LYS supplied in the GQM kit. Subsequent extraction of these 300-μL preparations, PCR and ELISA-based detection were performed as per the manufacturers' instructions.

To calculate the LoD when MRSA suspensions were absorbed onto Copan screening swabs prior to DNA extraction, 100 μL of each dilution above was adsorbed onto individual swabs. The swabs were allowed to dry for 30 min before vigorous vortexing in 300 μL of buffer Q-LYS and GQM processing.

Using the CFU/mL count determined above, the LoD of each reaction was determined, and the equivalent CFU/mL was calculated. LoD were recorded as the lowest concentration that produced a GQM assay positive reaction.

GQM assay inhibition by CA agar

To establish if culturing screening specimens on CA prior to GQM processing resulted in any PCR reaction inhibition, the following experiment was carried out (n = 3). A ten-fold dilution series of a 0.5 M^{c}Farland *S. aureus* ATCC 43 300 was made in 1-mL volumes of sterile saline. Forty microlitres of each dilution was added to 260 μL of GQM buffer Q-LYS. A duplicate set of dilutions was prepared. In the first set, sterile Copan swabs were rubbed on CA plates immediately before washing in each 300 μL dilution sample and processing by GQM. In the second set, sterile Copan swabs that had not been exposed to CA agar were washed in each 300-μL dilution and processed by GQM. The number of CFU per dilution was determined by spread plating (as for LoD above). LoD results from the two sets were compared, to determine if rubbing the swab on CA prior to PCR had an inhibitory affect.

GQM version 2.0

All tests were performed according to the manufacturer's instructions except that specimens were cultured on CA

prior to washing the swab in lysis buffer. The PCR amplification mix consisted of 35 μL primer nucleotide mix, 5 μL 10× polymerase buffer, 0.5 μL of 25 mM $MgCl_2$ 0.3 μL Fast start Taq (5 U per μL) (Roche, Basel, Switzerland), 5 μL DNA template and 4.2 μL water. The amplification protocol was according to the manufacturer's instructions.

Patient specimen collection and processing

Nasal and groin swabs were collected from patients on various hospital wards in a 700-bed tertiary referral hospital with endemic MRSA, over an 8-week period, as part of routine MRSA screening measures. Five hundred and forty specimens from 250 patients were collected and processed (270 groin and 270 nasal). Swabs were cultured on CA plates before processing by GQM. Discrepant results were further examined as follows. Swabs that were PCR positive but culture negative were cultured a second time, but were enriched overnight in 5 mL of tryptone salt broth 6% NaCl (37°C at 150 rpm in a shaking incubator) and subsequently plated on CA. Specimens that were PCR negative but culture positive were tested a second time by GQM PCR, using 5 μL of the 300 μL extracted specimens. Additionally, MRSA isolates recovered from discrepant specimens were tested by PCR in pure culture as per manufacturer's instructions.

Results

Analytical sensitivity and specificity

The GQM assay detected all MRSA strains in a collection representative of MRSA in Ireland since 1974 (n = 32). All eight MRCNS tested negative. Of seven GrMSSA strains tested, three tested positive by PCR and are considered false positives.

LoD assays

Using a saline dilution series and colony formation for calibration, when MRSA suspensions were used directly for DNA extraction and subsequent GQM amplification, the LoD was determined to be 704 CFU/GQM reaction (equivalent to a calculated 1.7×10^4 CFU/mL). As only 5 μL of the extracted 300 μL DNA preparations can be used as template in the GQM PCR reaction, this translates to an LoD per PCR of 11.8 CFU.

The LoD when MRSA suspensions were adsorbed onto Copan screening swabs prior to DNA extraction and subsequent GQM amplification was determined to be 1.4×10^3 CFU/swab, equivalent to a calculated 1.4×10^4 CFU/mL.

Chromogenic agar inhibits the Hain® GQM assay

In a GQM reaction where a swab was washed in GQM buffer Q-LYS containing a known number of MRSA cells, the lowest number of MRSA detected was 1.4×10^3 CFU/reaction. By contrast, where the swab was first rubbed on a CA plate and then washed in GQM buffer Q-LYS containing the same number of MRSA cells as above, the lowest number of MRSA detected was 5.6×10^3 CFU/reaction, suggesting that processing of swabs on CA prior to GQM PCR does have an inhibitory effect and increases LoD per reaction or CFU/mL by a factor of four.

Clinical specimens

Of 540 specimens processed 47/540 (8.7%) were both PCR and culture positive and 451/540 (83.5%) were negative by both methods. Discrepant results occurred with 42/540 (7.7%) specimens; four were PCR positive but culture negative and 38 were PCR negative but culture positive. All four patients with a positive PCR and culture-negative result had been decolonized and thus were considered true MRSA positives. The amended MRSA positive rate was therefore calculated as 51/540 (9.4%), with a discrepant rate of 38/540 (7.0%). For these remaining 38 discrepant results (PCR-negative/culture-positive specimens), PCR analysis of the prepared DNA extracted/lysis reactions was repeated and all were confirmed negative.

All 38 swabs were plated a second time on CA and subsequently enriched in Tryptic Soy Broth (TSB), for 24 h followed by plating on CA. Twelve grew after direct plating and enrichment, suggesting that the microorganisms were present on the swabs but were not detected by PCR. PCR on all 12 of these isolates (colony tested) were PCR positive, therefore these were considered true PCR false negatives in terms of the predictive value of the PCR assay.

Staphylococcus aureus was not isolated again after repeat culture with enrichment in TSB for the remaining 26/38 discrepant specimens. These were considered false negatives. The sensitivity, specificity, positive predicative value (PPV)

TABLE 1. Sensitivity, specificity, PPV and NPV of the Hain GQM assay

	PCR positive	PCR negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture positive	51	38	57	100	100	92
Culture negative[a]	0	451	–	–	–	–

PPV, positive predictive value; NPV, negative predictive value.
Note: [a]Four samples found to be culture negative, PCR positive were deemed to be false negatives, derived from decolonized patients, and are included as culture positives.

and negative predictive value (NPV) were calculated (Table 1).

Discussion

Conference proceedings by Eigner *et al.* [13] report on an evaluation of the GQM assay, reporting it as 100% sensitive, with a specificity of 99.4%, PPV 96% and NPV 100%, whereas Boegli-Stuber [14] report a GQM sensitivity of 91.3%. These results were promising, as GQM was 50% cheaper than other molecular methods and could provide a cost-effective alternative for rapid MRSA diagnostics. Additionally, this study showed that GQM detects SCC*mec* types I–V, whereas MSSA or coagulase-negative *Staphylococci* test negative. Our data support this, with SCC*mec* VI also being detected. However, three of seven GrMSSA strains tested here produced a GQM-positive, culture-negative result. These strains [12] harbour remnants of SCC*mec* DNA. As the majority of commercially available assays use PCR to detect SCC-associated sequences directly from clinical specimens, a PCR-positive/culture-negative result warrants caution and further investigation, e.g. culture on non-selective media for isolate characterization.

The LoD of GQM can be reported in a number of ways. Here we established the LoD of the complete GQM assay to be 704 CFU. This is the minimum number of MRSA cells required to give a positive hybridization signal when added to the GQM extraction mix. LoD can also be expressed as LoD per PCR and this has been calculated as 11.8 CFU per PCR. Many studies report LoD of rapid assays in CFU per mL or CFU per swab, thus our GQM LoD results can also be reported as 1.4×10^4 CFU/mL or 1.7×10^3 CFU/swab. By contrast, the LoD of other rapid methods has been reported as 6.1×10^2 CFU/mL or 5.8×10^1 CFU/swab for GeneXpert and 1.7×10^2 CFU/swab for routine MRSA chromogenic culture [4].

Processing clinical specimens on CA with subsequent GQM amplification, as described here, results in a discrepant rate of 7% (38 specimens were PCR negative, culture positive). Twelve of the 38 discrepant specimens reported as PCR negative/culture positive were culture positive both prior to GQM processing and when cultured a second time after discharge in the Q-LYS lysis buffer, suggesting that these were false negatives by GQM.

However, 26/38 specimens that were culture positive/PCR negative were not culture positive after a second culture attempt following discharge in the GQM buffer Q-LYS solution. Some possible explanations for these discrepancies include: (i) discrepancy due to this workflow, i.e. processing by culture before GQM removed all organisms from the swab, hence a GQM negative result; (ii) PCR inhibition resulted from rubbing the swab on a CA plate prior to GQM processing; (iii) the lysis reaction may have failed or may not have been optimal; and (iv) these 26 specimens were not detected as GQM positive because inoculum levels were below the LoD of the GQM assay. Calculations of the assay sensitivity depend on the interpretation of these findings. If we excluded the 26 GQM-negative, culture-positive specimens from sensitivity calculations, assuming that because of the work flow used all organisms had been removed by culture prior to PCR or that CA inoculation resulted in inhibition, a GQM sensitivity of 81% would have been achieved. Including the 26 specimens in the sensitivity calculation but assuming that all 26 specimens would have been PCR positive/culture positive if specimens had been processed by PCR first followed by culture would result in an improved sensitivity of 87%. However, the actual sensitivity determined here was 57%. The true value for the sensitivity value is at best between 81% and 87% but at worst between 57% and 81%, depending on the interpretation of the PCR-negative, culture-positive discrepant results. A full study to establish the diagnostic sensitivity of Hain GQM would be valuable.

We acknowledge the limitations in our evaluation, which include the number of specimens tested, confining the testing to nasal and groin specimens only (other licensed specimens include throat and wounds), and the taking of only one specimen per site for both PCR and culture.

Although an evaluation of two swabs per site, i.e. one for PCR and one for culture, might have led to fewer discrepant results, this represents considerable additional processing time and expense for routine diagnostic laboratories. Additionally, we did not want to alter the accredited method of culture as this was an evaluation in a routine diagnostic setting, thus minimizing any deviations from normal practice was important. Furthermore, discrepancies may still have occurred if two specimens had been taken, e.g. due to a greater load of MRSA being swabbed with the first specimen compared with the second. However, the additional expense and time incurred should be considered when evaluating new diagnostic methods.

In summary, whereas agreement of results occurred for 92.3% of specimens processed with culture first followed by PCR, the number of discrepant results and questions posed by these results suggest that culture followed by PCR using the one swab is not suitable for routine use. It is unclear if an alternative swab processing regime would overcome these shortcomings, but this may result in a reduced yield from culture. Consequently it is likely that

where both culture and PCR are being used, two swabs need to be taken with the resulting time and expense incurred.

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Transparency Declaration

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